



- day two, test samples (20  $\mu$ l) containing the test PRO polypeptide are added. On day five, the cells are fixed and then stained. An increase in ANP message can also be measured by PCR from cells after a few hours. Results are based on a visual score of cell size: 0 = no inhibition, -1 = small inhibition, -2 = large inhibition. A score of less than 0 is considered positive. Activity reference corresponds to phenylephrin (PE) at 0.1 mM, as a positive control. A score of 2 is considered very responsive. Assay media included: M199 (modified)-glutamine free,  $\text{NaHCO}_3$ , phenol red, supplemented with 100 nM insulin, 0.2% BSA, 5 mM creatine, 2 mM L-carnitine, 5 mM taurine, 100 U/ml penicillin G, 100  $\mu$ g/ml streptomycin (CCT medium). Only inner 60 wells are used in 96 well plates. Of these, 6 wells are reserved for negative and positive (PE) controls. Initially, quantitative PCR will be run in parallel to determine relative level of sensitivity to the visual scoring system.
- 10 PRO269 and PRO356 showed positive results in inhibition of heart adult hypertrophy in the above described assay.

### EXAMPLE 55

#### Stimulation of Endothelial Cell Proliferation

- This assay is designed to determine whether PRO polypeptides show the ability to stimulate adrenal cortical capillary endothelial cell (ACE) growth.

- Bovine adrenal cortical capillary endothelial (ACE) cells (from primary culture, maximum of 12-14 passages) were plated in 96-well plates at 500 cells/well per 100 microliter. Assay media included low glucose DMEM, 10% calf serum, 2 mM glutamine, and 1X penicillin/streptomycin/fungizone. Control wells included the following: (1) no ACE cells added; (2) ACE cells alone; (3) ACE cells plus VEGF (5 ng/ml); and (4) ACE cells plus FGF (5 ng/ml). The control or test sample. (in 100 microliter volumes), was then added to the wells (at dilutions of 1%, 0.1% and 0.01%, respectively). The cell cultures were incubated for 6-7 days at 37°C/5%  $\text{CO}_2$ . After the incubation, the media in the wells was aspirated, and the cells were washed 1X with PBS. An acid phosphatase reaction mixture (100 microliter: 0.1M sodium acetate, pH 5.5, 0.1% Triton X-100, 10 mM p-nitrophenyl phosphate) was then added to each well. After a 2 hour incubation at 37°C, the reaction was stopped by addition of 10 microliters 1N NaOH. Optical density (OD) was measured on a microplate reader at 405 nm.

- The activity of a PRO polypeptide was calculated as the fold increase in proliferation (as determined by the acid phosphatase activity, OD 405 nm) relative to (1) cell only background, and (2) relative to maximum stimulation by VEGF. VEGF (at 3-10 ng/ml) and FGF (at 1-5 ng/ml) were employed as an activity reference for maximum stimulation. Results of the assay were considered "positive" if the observed stimulation was  $\geq$  50% increase over background. VEGF (5 ng/ml) control at 1% dilution gave 1.24 fold stimulation; FGF (5 ng/ml) control at 1% dilution gave 1.46 fold stimulation.

PRO179, PRO212, PRO1075, PRO1154, PRO1244, PRO1286, and PRO1303 assayed "positive" as shown in TABLE 6 below: